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Sister Chromatid Exchange in a Marine Polychaete Exposed to a Contaminated Harbor Sediment

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16. ABSTRACT

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SISTER CHROMATID EXCHANGE IN A MARINE POLYCHAETE EXPOSED TO A CONTAMINATED HARBOR SEDIMENT*

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INTRODUCTION

It is the policy of the United States to regulate disposal of all types of materials into ocean waters and to prevent or strictly limit disposal of any material which would adversely affect human health, welfare, the marine environment, or ecological systems. This policy is implemented through the issuance of permits as defined by the U.S. Environmental Protection Agency (U.S. EPA) in the regulations for the Marine Protection, Research, and Sanctuaries Act (Public Law 92-532).

In 1977, the U.S. EPA published final regulations and criteria for ocean dumping (U.S. Environmental Protection Agency, 1977). Section 227.6 listed constituents which were prohibited as other than trace contaminants. For the first time, this category included "known carcinogens, mutagens, or teratogens or materials suspected to be carcinogens, mutagens, or teratogens by responsible scientific opinion."

At present, however, no genotoxic tests are included in permit programs designed to manage waste disposal in estuarine, coastal, and oceanic environments. Short-term tests are needed to detect mutagenic activity in complex mixtures. Long-term tests are needed to investigate possible effects of somatic and germinal mutations on populations of marine species.

Environmental samples can be analyzed for the presence of genetic toxicants but this provides no information on the bioavailability of sediment sorbed compounds. Furthermore,

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since many classes of compounds are genetically active, the number of analyses would be extensive. A more direct approach is to look for genetic damage in exposed biota. Because cytogenetic techniques are sensitive and relatively simple, it has been recommended that genetic damage in marine organisms be determined by observing their chromosomes directly (International Atomic Energy Agency, 1979; Kligerman, 1980).

The cytogenetic technique of choice is sister chromatid exchange (SCE). The SCE response has been recommended for environmental application by the U.S. EPA's Gene-Tox Program (Latt et al., 1981). The application of SCE to polychaetes has created a new tool that is both relevant and practical for the study of genetic problems in marine environments (Pesch et al., 1981). With the *in vivo* SCE assay, complex wastes can be tested under conditions which better represent actual environmental situations.

This report evaluates the use of SCE to measure DNA-damaging activity associated with contaminated dredged material. The primary objectives were to test the applicability of the SCE technique and to field verify any responses observed in the laboratory. The SCE technique was applied to *Nephtys incisa*, an infaunal polychaete dominant in the benthic community of Long Island Sound. The SCE response was measured in *N. incisa* exposed in the laboratory to suspended and bedded phases of sediments dredged in Black Rock Harbor (BRH), Connecticut, and in *N. incisa* sampled along a transect of stations at the Central Long Island Sound (CLIS) site where BRH material was disposed (Fig. 1).

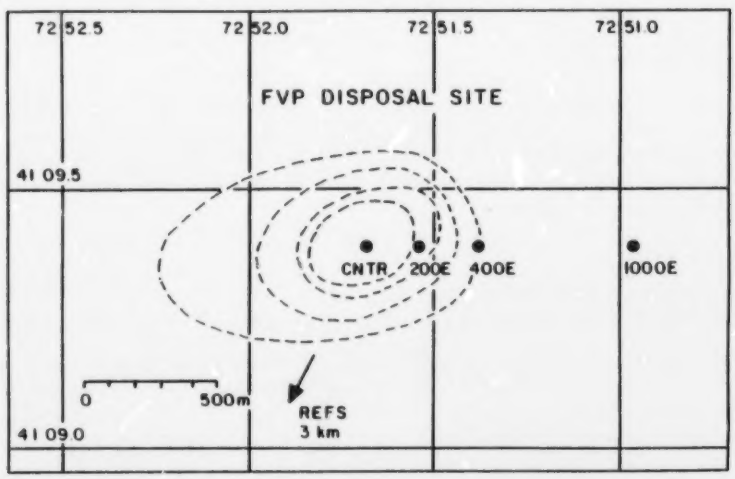

Figure 1. FVP disposal site and station locations.

MATERIALS AND METHODS

Laboratory Methods

Two sediments were used to conduct laboratory tests. Reference sediment (REF) was collected from the Reference site (Fig. 1) in Long Island Sound. Prior to dredging, contaminated sediment was collected from BRH. Both sediments were press sieved and refrigerated (4°C) until used.

Nephtys incisa for laboratory studies were collected at the field Reference site. Sediment containing *N. incisa* was brought to the laboratory where it was sieved, and the worms were picked and sorted by size. Tests were conducted with juvenile individuals 0.5 to 1.5 cm in length. These individuals were placed in REF sediment, in flowing seawater, and acclimated at a rate of l°C per day to 20°C. They were fed powdered prawn flakes, *ad libitum*, during this period.

A sediment exposure system continuously maintained and delivered both test sediments (as 3% slurries) to the exposure chambers after mixing the slurries with seawater to provide the desired concentrations for the bioassays. Narragansett Bay seawater filtered (to 15 microns) through sand filters was used. Actual concentrations of suspended sediments in the test chambers were determined periodically by dry weights (Lake et al., 1985).

For the laboratory exposure experiment a randomized complete block design was used with 3 replicates (blocks). Each replicate contained four exposure regimes. Each exposure regime had a single test chamber with 15 organisms. Due to limitations of space and availability of animals, the 3 replicates were run at successive times. Exposure conditions for the bedded phase portion of the suspended particulate tests were either 100 percent REF or 100 percent BRH. These two bedded phase exposure conditions in combination with the two suspended sediment exposures, REF or BRH at 200 mg/l (dry weight), gave a total of 4 treatments. The treatment with REF sediment in both bedded and suspended phases served as the control. The worms were fed prawn flakes (ADT-Prime, Aquatic Diet Technology, Brooklyn, NY) in a suspension of seawater, daily during each test. All tests were conducted with sand-filtered Narragansett Bay seawater at 20°C and approximately 30 g/kg salinity. Seawater flow rates were about 35 ml/min. The photoperiod was a 14:10 hr light-dark cycle.

Field Methods - Organism Collection and Holding

Nephtys incisa for field studies were collected at stations REFS, lOOOE, 400E, and 200E (Fig. 1). Station locations at the study site were marked with buoys for the duration of this project. While the boat was anchored, a Smith-McIntyre grab sampler was used to collect bottom sediments. These sediments were wet sieved on deck and organisms were collected. Specimens for SCE were returned to the laboratory for processing.

Chromosome Labeling - Laboratory and Field

To facilitate SCE observations, chromosomes were labeled with the base analog 5-bromodeoxyuridine (Brdu). Because the labeling phase must include two cell cycles, the time needed varies according to growth rate. Therefore, this time may differ with species and age of the test organism. In this study, these times were determined empirically.

The labeling phase was done subsequent to the laboratory exposure or directly upon return from field sampling. The only difference between the labeling of laboratory worms and field worms was the temperature of the seawater. Laboratory worms were labeled at 20°C, while the field worms were labeled at ambient seawater temperature which ranged from 0 to 20°C depending on season. In both cases the worms were exposed to 3 mg Brdu/L of seawater for 10 to 20 days in the dark. Colchicine (0.05 percent) was added to the seawater for the last 15 hrs of the labeling period to arrest cell division at metaphase.

Slide Preparation and Staining

The procedure for slide preparation was adapted from Kligerman and Bloom (1977). Slides were stained according to a procedure recommended by Bloom (personal commun., 1981, Cornell University, Ithaca, NY). Details may be found in Pesch et al. (1985). Observations were made with a compound microscope at 1250x, oil immersion.

Data Collection and Analysis

The SCE observations were made on 25 second-division (metaphase stage) cells for each treatment unless otherwise noted. Cells were selected under low power, then counted under high power. For each treatment, the individual worms were screened sequentially. Counting continued until a total of 25 cells were counted regardless of the number of individual worms. This assumes that organism-to-organism variance was small compared to within organism variance. This assumption has been tested for these data and found to be true.

The data were examined to see whether criteria were met for parametric statistical analysis or whether data transformation was necessary. All of the SCE data in this report were transformed to log 10 (SCE/chromosome + 0.1) prior to statistical analysis. The laboratory data were analyzed by two-way analysis of variance for a randomized complete block design (Snedecor and Cochran, 1980). All other comparisons were analyzed by one-way analysis of variance.

RESULTS

Laboratory Results

During the three *N. incisa* laboratory tests for SCE response the exposure systems maintained the suspended sediment concentrations at 200 ± 70 mg/L. Temperature and salinity values were stable at approximately 20°C and 30 g/kg, respectively. Dissolved oxygen concentrations were checked once during each experiment and never differed from saturation.

The replicated and pooled particulate phase/solid phase test data are presented in Table 1. In the pooled data, the SCE frequencies for the B/R treatment (numerator = suspended phase, denominator = bedded phase) were significantly higher ($P = 0.053$, approximately 50 percent higher) than any other treatment. There were no significant differences among the other treatments.

Johns and Gutjahr-Gobell (1985) studied the impact of BRH sediment on the bioenergetics of *N. incisa*. These studies were conducted simultaneously in the same exposure system as the SCE studies. Data for net growth efficiency and SCE are compared in Figure 2. They

Table 1. Sister Chromatid Exchange/Chromosome Response in *Nephtys incisa* to Particulate Phase/Solid Phase Exposure to BRH Sediment

Treatment	Replicate 1	Replicate 2	Replicate 3	Pooled Data
		Log Transformed Data		
B/R*	−0.450 ± 0.050**(25)†	−0.414 ± 0.055(12)	−0.194 ± 0.056(19)	−0.355 ± 0.035(56)
B/B	−0.543 ± 0.050(26)	−0.513 ± 0.054(15)	−0.449 ± 0.053(25)	−0.500 ± 0.031(66)
R/B	−0.608 ± 0.056(25)	ND‡	−0.403 ± 0.055(24)	−0.508 ± 0.041(49)
R/R	−0.494 ± 0.145 (7)	−0.512 ± 0.049(25)	−0.482 ± 0.123 (9)	−0.502 ± 0.046(41)
		Untransformed Data		
B/R	0.306 ± 0.038(25)	0.321 ± 0.053(12)	0.627 ± 0.078(19)	0.418 ± 0.033(56)
B/B	0.246 ± 0.050(26)	0.239 ± 0.037(15)	0.319 ± 0.049(25)	0.272 ± 0.028(66)
R/B	0.200 ± 0.039(25)	ND	0.376 ± 0.065(24)	0.286 ± 0.038(49)
R/R	0.323 ± 0.105(7)	0.252 ± 0.034(25)	0.343 ± 0.115(9)	0.284 ± 0.037(41)

Note: Data expressed as log 10 of means.

* R = reference sediment, B = Black Rock Harbor sediment, numerator = suspended phase, denominator = bedded phase.

** Standard error of the mean.

† Number in parenthesis is sample size (N).

‡ ND - no data.

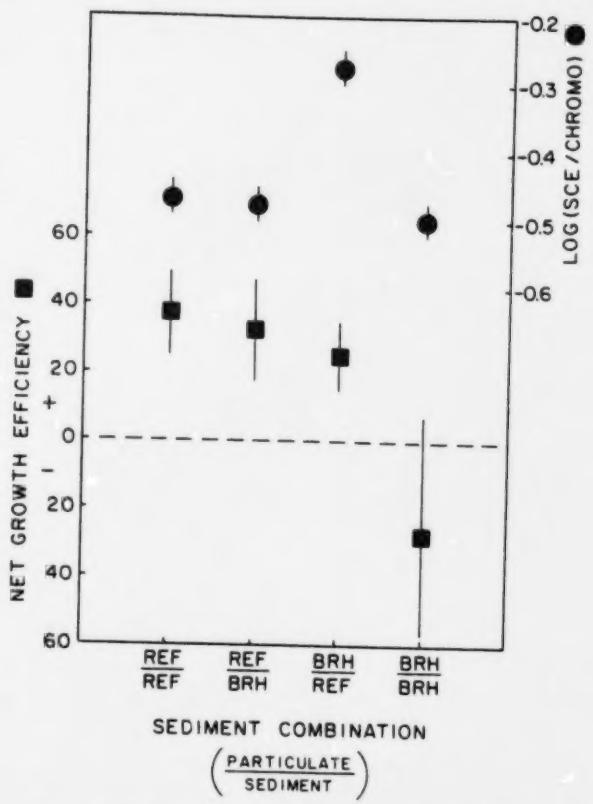

Figure 2. A comparison of SCE and net growth efficiency responses measured in the same experiments suggesting the dependence of the SCE response on the physiological condition of the worms.

found that the presumably worst-case treatment (B/B) caused *N. incisa* to lose weight during the tests, whereas the B/R treatment was not significantly different from the control treatment (R/R) for any of the measures of energetic effects. The worms in the worst case treatment (B/B) were inactive, did not eat, and had reduced metabolic activity. These responses, in effect, isolated these worms from exposure. The worms in the B/R treatment did well metabolically and thus were susceptible to the BRH exposure. The worms in the R/B treatment physically isolated themselves from the exposure by moving from the BRH bedded phase sediment into the surficial layer of REF sediment deposited within the exposure chamber during the experiments.

Field Results

SCE observations were made on juvenile (young of the year) *N. incisa* collected at stations 200E, 400E, 1000E and REFS (Fig. 1). Data are available for three sampling dates: June 6, 1983 (3 weeks post-disposal), August 26, 1983 (14 weeks post-disposal), and December 13, 1983 (28 weeks post-disposal). These data are summarized in Table 2. Two patterns are clear. First, the SCE response is low in June, rises significantly in August, and declines in December. Second, there are no differences among stations on any given date. The pattern of increase in the August data is consistent at all stations. During the cruise for the August field survey, worms were collected for a laboratory replicate test. These worms, collected at the REFS site, were used for replicate number 3 in Table 1. These data are compared graphically in Figure 3.

Table 2. SCE/Chromosome Response in *Nephtys incisa* Sampled at the FVP Biological Effects Stations in CLIS

	Sampling Time Post-disposal (weeks)		
Station	June 1983 3	August 1983 14	December 1983 28
	Log Transformed Data		
200E	−0.749 ±0.029*(2)**	−0.252 ±0.066(12)	−0.404 ±0.205 (4)
400E	−0.603 ±0.029 (2)	−0.203 ±0.049(15)	−0.483 ±0.080 (7)
1000E	−0.705 ±0.295 (3)	−0.169 ±0.049(25)	−0.337 ±0.079 (5)
REFS	−0.603 ±0.054 (4)	−0.200 ±0.050(14)	−0.499 ±0.053(12)
	Untransformed Data		
200E	0.079 ±0.012(2)	0.532 ±0.092(12)	0.406 ±0.156(4)
400E	0.150 ±0.017(2)	0.582 ±0.074(15)	0.265 ±0.073(7)
1000E	0.222 ±0.222(3)	0.658 ±0.060(25)	0.391 ±0.088(5)
REFS	0.155 ±0.029(4)	0.584 ±0.074(14)	0.247 ±0.052(12)

Note: Data expressed as log 10 of means.
* Standard error of the mean.
** Number in parenthesis is sample size (N).

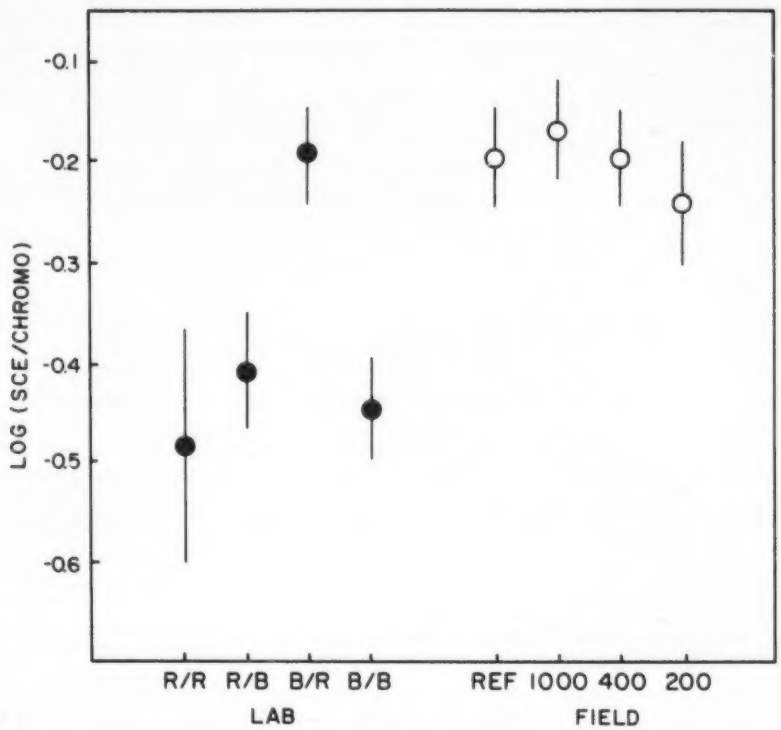

Figure 3. Comparison of laboratory and field data for SCE response in *Nephtys incisa*. Worms for the laboratory test were collected at the Reference Station during the cruise for the field observations (14 weeks post-disposal). All field data were significantly higher (2x) than laboratory controls (R/R). The laboratory B/R treatment data were the same as all the field data.

DISCUSSION

Laboratory Studies

The Gene-Tox panel recommended criteria for negative/positive conclusions for the SCE response (Latt et al., 1981). A weakly positive SCE response was classified as representing a statistically significant increase in SCE over baseline. A strongly positive SCE response was classified as representing at least a twofold SCE increase. If these decision criteria are applied to the results of the laboratory BRH experiment, the overall result with replicates pooled is a weakly positive SCE response in the B/R treatment. If replicate test

three (Table 1) is considered alone, the SCE response in the B/R treatment represents a twofold increase over the response in the R/R treatment. This represents a strongly positive SCE response.

The increased frequency of SCE observed in *N. incisa* is consistent with responses of the Ames test to extracts of BRH sediment. Whole extracts of BRH sediment elicited a dose response with *Salmonella* strain TA100 in the presence of S-9. This means that the mutation was a base-pair substitution and that the mutagens in BRH sediment needed to be metabolized before being reactive (Lee and Senecal, unpublished data generated under an EPA Cooperative Agreement with the University of Rhode Island #CR-812807-01-0). Based upon current understanding of mutagenesis, the positive results with the Ames test and the SCE response in *N. incisa* suggest a tumorigenic potential for BRH sediment. This is supported by Gardner et al. (1986) who report tumor development in American oysters and winter flounder exposed to BRH sediment under laboratory and field conditions.

The chemical composition of BRH sediment was reported by Rogerson et al. (1985). Many organic chemicals found in three standard solvent fractions of BRH sediment, and some selected inorganic compounds also identified in the sediment have been reported to be genotoxic. The first fraction was characterized as nonpolar and contained PCBs, saturated aliphatic hydrocarbons and cycloalkanes, dichlorodiphenyl-dichloroethylene (DDE), and some two-ring aromatic hydrocarbons (acenaphthene, acenaphthylene, biphenyl, and naphthalene). The weight of the evidence suggests that in general, PCBs are not genotoxic (Schoeny, 1982; International Agency for Research on Cancer, 1978, 1982b). PCBs may, however, contribute indirectly to the *in vivo* genotoxicity of promutagens by inducing MFO system enzymes important in metabolizing promutagens to active forms (Ramel, 1975). There is no evidence that the aliphatic hydrocarbons or their cyclic counterparts, the cycloalkanes, are genotoxic; however, there is a paucity of data in the literature. DDE is reported in one study (Sina et al., 1983) to induce DNA single strand breaks in rat hepatocytes, and acenaphthylene is reported to induce mutation in *Salmonella typhimurium* (Kaden et al., 1979). Many other studies, however, report negative results for these and other compounds in this solvent fraction (Mamber et al., 1983, 1984; McCann et al., 1975; Moriya et al., 1983; Mitchell et al., 1983; Zimmermann et al., 1984).

The second fraction was a slightly polar aromatic hydrocarbon fraction. For BRH sediment, this fraction contained many polycyclic aromatic hydrocarbons (fluorene, phenanthrene, dibenzothiophene, fluoranthene, pyrene, chrysene, benzanthracene, benzo[a]pyrene, benzo[e]pyrene, perylene, and various alkylated derivatives of these compounds, as well as benzoperylene, dibenzanthracene, coronene, and dibenzochrysene. Dichlorodiphenyltrichloroethane (DDT), chlordanes (hepta, octa, and nonachlor), and ethylan were also found in this fraction. Several of these compounds have been reported to be genotoxic in a number of short-term assays, usually following metabolic activation (International Agency for Research on Cancer, 1983). Genotoxic effects have been reported for ten-second fraction compounds (fluorene, pyrene, benzanthracene, chrysene, benzo[a]pyrene, benzo[e]pyrene, perylene, benzoperylene, dibenzanthracene, and coronene), although the evidence for three of these chemicals (perylene, benzoperylene, and coronene) is not sufficient to justify a genotoxic classification (International Agency for Research on Cancer, 1983). There is, however, limited or sufficient evidence that pyrene, benzanthracene, chrysene, benzo[a]pyrene, benzo[e]pyrene, and dibenzanthracene induce SCE in several *in vitro* or *in vivo* assay systems (International Agency for Research on Cancer, 1983). There is limited evidence that chlordane is genotoxic (International

Agency for Research on Cancer, 1979) and substantial evidence that DDT is not (International Agency for Research on Cancer, 1982b).

The third fraction was more polar and contained ketones, quinones, carbazoles, and phthalates. Evidence for the genotoxicity of these chemicals is limited. Natural quinones form a large class of colored pigments, occurring mainly in higher plants and some microorganisms, particularly the lower fungi. Several natural anthraquinones have been reported to be strongly mutagenic in *Salmonella* (strain TA2637) with metabolic activation (Tikkanen et al., 1983), possibly via the generation of active oxygen (Brown, 1980; Chesis et al., 1984; Lesko and Lorentzen, 1985; Smith, 1985). In addition, several substituted anthraquinones have recently been reported to induce chromosomal aberrations and SCEs in cultured mammalian cells (Nishio et al., 1982). Some substituted carbazoles have been evaluated for genotoxic effects, primarily in procaryotic organisms, and reported to be genotoxic (International Agency for Research on Cancer, 1983). Phthalates have been studied extensively and are not genotoxic (International Agency for Research on Cancer, 1982a; Butterworth et al., 1984).

Potential contributions from inorganic compounds to the genetic effects observed in *N. incisa* must also be considered. From the genetic viewpoint, the most important inorganic elements identified in BRH sediment were arsenic, cadmium, chromium, copper, lead, manganese, and nickel. Concentrations of cadmium, chromium, and copper were particularly high, exceeding reference sediment concentrations by factors of 100, 30, and 46 respectively (Rogerson et al., 1985). Although only hexavalent chromium has been reported to consistently induce gene mutations in procaryotic and eucaryotic test systems (Rossman, 1981), other inorganic compounds have been reported to induce a variety of chromosomal effects, including SCE. Arsenic, cadmium, chromium, lead, nickel, and zinc have been reported to induce chromosomal aberrations in animals, plants, and cultured cells (Bianchi et al., 1983; Garrett et al., 1984; International Agency for Research on Cancer 1980; Ma et al., 1984; Ochi et al., 1984), whereas arsenic, chromium, and nickel have also been reported to induce SCE (Bianchi et al., 1983; Garrett et al., 1984; Newman et al., 1982). In addition, arsenic, copper, and manganese have recently been reported (Rossman and Molina, 1986) to be comutagens by enhancing the mutagenic activity of known mutagenic agents.

In summary, these sediments contain many polynuclear aromatic hydrocarbons in the second fraction, possible substituted anthraquinones and carbazoles in the third fraction, and several inorganic substances, all of which are capable of inducing SCE. The presence of these constituents predicts that this response will occur in organisms exposed to BRH sediment.

Field Studies

There are two clear patterns in the SCE data from the field studies. First, the SCE response is low in June 1983, rises significantly in August 1983, then declines in December 1983. Second, there are no differences among stations on any given date. If the Gene-Tox decision criteria are applied, the June to August increase represents a strongly positive SCE response. This increase in SCE frequency is not a seasonal effect. The data in Figure 3 demonstrate this clearly. Worms for the laboratory test were collected at the Reference Station during the cruise for the field observations. The laboratory control worms (R/R) exhibit an SCE frequency one-half that of the field REFS site worms. The only difference between these two samples of worms was the length of time in the laboratory. Ambient temperature in

CLIS at the time of collection was 20.8°C. The worms were held at 20°C in the laboratory. Salinities were comparable also.

The August field data (Fig. 3) indicate an increased SCE frequency. The SCE frequency declines when the worms are held in clean laboratory conditions. The SCE frequency in the laboratory held worms returns to field levels when the worms are exposed to BRH sediments.

The SCE response at all field stations in August implies exposure to contaminated sediments at all field stations. There is evidence to support this implication. A physical model based upon *in situ* current velocity data and records of disposal events permitted tracking single disposal plumes over time to examine areas of impact (Munns and Paul, 1987). Model predictions compare favorably with near-continuous turbidity measurements obtained from *in situ* instrumentation. Long-term, time-averaged concentrations of contaminated sediment estimated by the model at selected stations agreed with concentrations estimated from tissue residues of *Mytilus edulis* (Munns and Paul, 1987) deployed 1 meter above the bottom at those stations. Model predictions also agree with chemical concentrations measured in the water at 1 meter above the bottom at these stations. The model describes both near (1 km) and far (1 km) field dispersal of dredged material disposed of at this site in Long Island Sound (Munns and Paul, 1987).

To understand why the SCE response in *N. incisa* had not been initiated by 3 weeks post-disposal and then why, when it was initiated, the response was the same throughout the entire CLIS study area, we need to examine the biology of the SCE response. An SCE is a visual consequence of an effect directly on the DNA. If there is genotoxicity associated with BRH sediments, a likely cause is the polynuclear aromatic hydrocarbons (PAHs). These compounds are not direct-acting mutagens. They need to be metabolized to reactive intermediates. A key enzyme system in this metabolism is the cytochrome P-450 dependent mixed function oxygenase system (MFO). Fries and Lee (1984) report an induction time of 4 to 8 weeks for the MFO system in the marine polychaete *Nereis virens* exposed to benzo(a)pyrene [B(a)P]. The SCE observations were made on young of the year *N. incisa*. *Nephtys incisa* spawns in CLIS during April and May (Carey, 1962). The larvae settle from their planktonic stage on to the sediments during May and June (Zajac and Whitlatch, 1985). Therefore, the SCE data from field worms collected 3 weeks post-disposal may well be from worms whose MFO system had not been activated; and, therefore, we would not expect to see an increase in the SCE response.

The tissue concentrations of PCBs and PAHs in *N. incisa* during the summer of 1983 indicate that the MFO system in the exposed worms had been activated by 14 weeks post-disposal. PCB tissue concentrations reached a peak in September, 4 months post-disposal, indicating a continuous exposure to contaminated sediments during this period. In contrast, the highest PAH tissue concentrations occurred in July, only 2 months post-disposal. These data suggest that metabolism of PAHs was induced and was causing a sharp decline in tissue PAH concentrations despite continuous exposure to these compounds. Presumably, by 14 weeks post-disposal the worm MFO system had been induced, resulting in a significant SCE response.

When the SCE response occurred, it occurred to the same extent at all four biological stations (Table 2). To understand this, we need to examine the nature of SCE responses to PAHs and complex mixtures. In both cases, the usual response is about a doubling of SCEs over background and then no further increase. The biological explanation of this "plateau" is not clear, but it has been observed in widely different test systems. For B(a)P, this "plateau" has

been observed *in vitro* in human cells (Juhl et al., 1978; Abe, 1984; Hopkin, 1984), in Chinese hamster cells (Pal et al., 1978; Bloom, 1984; Abe, 1984; Hopkin, 1984), and in rat cells (Abe, 1984). The "plateau" effect has been observed in *in vivo* studies involving rabbits (Takehisa and Wolff, 1978), in a marine fish (Stromberg et al., 1981), and in a polychaete (Pesch et al., 1981). In two separate studies involving exposure of a freshwater fish, *Umbra pygmae*, to a complex mixture (polluted Rhine River water), a two to threefold increase in SCEs was found (Hooftman and Vink, 1981; Alink et al., 1980).

In the present study, SCE frequencies in worms from the REFS site increased by a factor of 3.8 (Table 2) between June 6 (3 weeks post-disposal) and August 26 (14 weeks post-disposal). The SCE frequencies in worms from the other three FVP stations increased to the same extent during this period, even though tissue residue analyses indicate they had been exposed to much higher BRH concentrations. The simplest explanation for this response pattern is that the "plateau" had been reached at the REFS station; therefore, the SCE response was insensitive to further increase at the other three stations, despite higher exposure concentrations of BRH. The laboratory experiments support the hypothesis of a "plateau". The greatest increase in SCE frequency observed in the laboratory (treatment B/R in Rep. 3, Table 1), at exposures higher than estimated for the field, was statistically the same as the August 26 field data. An SCE frequency of about 0.6 SCE/chromosome appears to be the "plateau" for *N. incisa* exposed to BRH sediments, and by inference the exposure levels needed to reach this "plateau" were present throughout the entire CLIS study area during the summer of 1983.

SUMMARY

Contaminated sediment dredged in Black Rock Harbor, CT, elicited increased SCE frequencies in a marine polychaete, *Nephtys incisa*, under controlled laboratory exposures. The SCE response was measured in *N. incisa* sampled along a transect of stations in the field at the site where this contaminated sediment was disposed. The SCE frequencies increased significantly in field *N. incisa* by 14 weeks post-disposal. The increased SCE frequencies declined when the *N. incisa* were held in clean, laboratory conditions. The SCE frequencies increased to field levels when the laboratory held *N. incisa* were exposed to BRH sediment. The increased SCE frequencies associated with exposure to BRH sediment indicate a potential problem and underscore the need for genotoxicity testing in ocean disposal permit programs.

DISCLAIMER

The views expressed in this article are those of the authors and not necessarily those of the U.S. Environmental Protection Agency.

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